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Multiple Pathways in the Decision to Flower: Enabling, Promoting, and Resetting

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INTRODUCTION

At a certain point in their life cycle, annual plants undergo a major developmental transition and switch from vegetative to reproductive development. This process is rarely reversible, and ensuring that the timing of this transition is optimal for pollination and seed development is a major factor in reproductive success. Physiological and genetic analysis of flowering has shown that multiple environmental and endogenous inputs influence the timing of the switch. The molecular identity of these different inputs is being dissected using molecular genetic approaches in Arabidopsis. The multiple pathways quantitatively regulate an overlapping set of common targets, the floral pathway integrators, whose activities convert the shoot apical meristem to a reproductive fate. An emerging idea is that changing the predominance of these input pathways could account for much of the plasticity and diversity of flowering time control within and between plant species (Simpson and Dean, 2002). During the last few years, the data relevant to a molecular understanding of flowering time control have increased rapidly, making it unwieldy to comprehensively review the field. In addition, there have been numerous and excellent recent reviews on various aspects of flowering time control (Mouradov et al., 2002; Ratcliffe and Riechmann, 2002; Simpson and Dean, 2002; Henderson et al., 2003; Yanovsky and Kay, 2003). To complement these, we have chosen, in this review, to group genes involved in flowering time into pathways that enable the floral transition and those that promote it. We also discuss the role of genes defined through early-flowering mutants. Are they specific floral repressors or more global regulators needed to reset patterns of gene expression?

INPUT PATHWAYS REGULATE COMMON TARGETS

Genetic analysis of a large number of Arabidopsis flowering time mutants has led to a model describing an integrated network of pathways that quantitatively control the timing of the floral transition. We present a version of this model (Figure 1) in which we divide the floral pathways into those that enable the floral transition and those that promote it. The different pathways that promote the floral transition include those that mediate photoperiod, hormone biosynthesis and signaling, light quality, and ambient temperature cues. They activate the expression of genes that cause the floral transition. These have been called ''floral pathway integrators'' and include *FLOWERING LOCUS T* (*FT*), *LEAFY* (*LFY*), and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*), also known as *AGAMOUS-LIKE 20* (*AGL20*) (Nilsson et al., 1998; Kardailsky et al., 1999; Kobayashi et al., 1999; Blázquez and Weigel, 2000; Lee et al., 2000; Samach et al., 2000). By contrast, the pathways that enable the floral transition regulate the expression of floral repressors. These antagonize the pathways described above that promote the activation of the floral pathway integrators. We view the enabling pathways as regulating ''meristem competence'' (Bernier, 1988). High levels of the floral repressors keep the meristem ''blind'' to promotive floral signals.

PATHWAYS THAT ENABLE THE FLORAL TRANSITION

We define the enabling pathways as those that regulate repressors whose function is to antagonize the activation of the floral pathway integrators. Some activities decrease and others increase the effectiveness of the repressors (Figure 2). The pathways that regulate the floral repressor *FLOWERING LOCUS C* (*FLC*) are the most well characterized in this group, but genetic analysis suggests that *TERMINAL FLOWER1* (*TFL1*; Ruiz-García et al., 1997), *SHORT VEGETATIVE PHASE* (*SVP*; Hartmann et al., 2000), *TARGET OF EAT1/2* (*TOE1/2*; Aukerman and Sakai, 2003), as well as *FLC* homologs (Ratcliffe et al., 2001, 2003; Scortecci et al., 2001, 2003) may be other repressors that influence enabling activity.

This concept of pathways that enable the floral transition emerged from the analysis of double and triple combinations of mutants from the classically defined photoperiod, gibberellin (GA), and autonomous pathways (Reeves and Coupland, 2001). Single autonomous pathway mutants (*fca-1*), which had high levels of *FLC*, flowered much later than single photoperiod pathway (*constans-2* [*co-2*]) or GA pathway (*ga1-3*) mutants under long-day conditions. Under the same conditions, *co-2 ga1-3* double mutants (in which FCA is active) flowered extremely late, if they flowered at all, and were later flowering than *co-2 fca-1* and *ga1-3 fca-1*. Therefore, FCA activity by itself has little promotive effect on flowering time. However, if FCA activity is absent and FLC levels are high, the plant does not respond as well to promotive flowering signals.

The pathways that enable the floral transition could be likened to the control of a sluice gate in a lock on a canal. The plant is like a boat waiting to move from one lock to the next (vegetative to reproductive development). The floral repressors act as the gate

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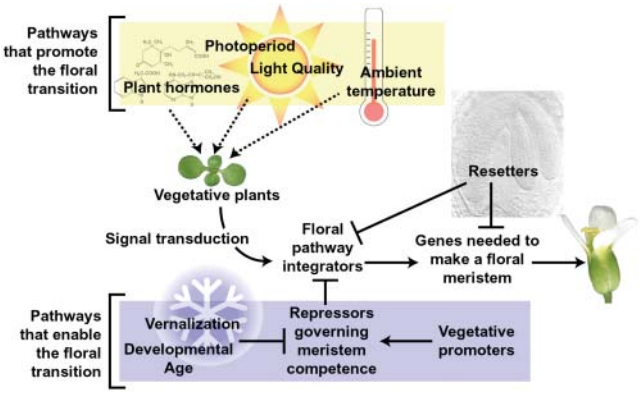

Figure 1. Pathways That Enable or Promote the Floral Transition Determine Flowering Time.

The different pathways are grouped into those that promote and those that enable the floral transition. The enabling pathways regulate the ability of the meristem to respond to floral promotive signals from different environmental and endogenous cues.

itself, inhibiting the passage of water (floral promotion) into the lock. However, there are multiple controls that can open or close the sluice gate, and their combined action determines how high the gate rises and thus how much water is permitted to flow through. It is the water itself that allows the boat to move on to the next lock, but how long the water takes reach the right level is determined by the control of the sluice gate.

The Floral Repressor *FLC*

FLC is a MADS box transcriptional regulator, expressed predominantly in shoot and root apices (Michaels and Amasino, 2000), that acts to quantitatively repress flowering (Michaels and Amasino, 1999a; Sheldon et al., 1999) through repression of the floral pathway integrators *FT*, *SOC1*, and *LFY* (Nilsson et al., 1998; Kardailsky et al., 1999; Kobayashi et al., 1999; Blázquez and Weigel, 2000; Lee et al., 2000; Samach et al., 2000). *FLC* plays a central role in vernalization requirement and response. Arabidopsis vernalization-responsive mutants and accessions that show a strong vernalization requirement have increased *FLC* RNA and protein levels, whereas vernalized plants have reduced *FLC* levels (Sheldon et al., 2000b; Michaels and Amasino, 2001; Rouse et al., 2002). Overexpression of *FLC* results in very delayed flowering in Landsberg *erecta* (L*er*), which is vernalization insensitive, but in C24 some plants flower late and others flower early (Michaels and Amasino, 1999a; Sheldon et al., 1999).

Analysis of natural variation in different Arabidopsis accessions has shown that allelic variation at *FLC* contributes to flowering time (Michaels and Amasino, 2000; Schlappi, 2001; Gazzani et al., 2003). In the case of L*er*, this appears to be the result of altered regulation of the gene rather than of different FLC protein activities. The *FLC* allele in Columbia is much stronger than that of L*er*, and this is correlated with the presence of a nonautonomous *Mutator*-like transposable element positioned in the first intron of *FLC* in L*er*, a region known to be required for *FLC* regulation (Sheldon et al., 2002; Gazzani et al., 2003; Michaels et al., 2003b).

There are five close homologs of *FLC* in the Arabidopsis genome called *MADS AFFECTING FLOWERING1* (*MAF1*) to *MAF5* (Ratcliffe et al., 2001, 2003) (MAF1 is also called FLOWERING LOCUS M [FLM] [Scortecci et al., 2001]). *MAF1* to *MAF4* appear to act as floral repressors whose expression is affected only slightly, if at all, by vernalization. *MAF5* expression is upregulated by vernalization, but the role it plays in determining flowering time is unclear (Ratcliffe et al., 2003).

The Floral Repressors *TFL1*, *SVP*, and *TOE1/2*

TFL1 encodes a protein that is similar to animal Raf kinase inhibitors and is highly homologous with *FT* (Bradley et al., 1997; Ohshima et al., 1997; Kardailsky et al., 1999; Kobayashi et al., 1999). In contrast to the floral promoting activity of FT, TFL1 extends the vegetative growth phase and maintains the indeterminate nature of the inflorescence (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Bradley et al., 1997). These activities are thought to result from *TFL1* both delaying the upregulation of *LFY* and *APETALA1* (*AP1*)/*CAULIFLOWER* (*CAL*) and also preventing a response to these genes in the inflorescence meristem (Bradley et al., 1997; Ratcliffe et al., 1998). Conversely, LFY and AP1/CAL inhibit *TFL1* expression in the floral meristems produced on the periphery of the inflorescence meristem (Liljegren et al., 1999; Ratcliffe et al., 1999). The relative activity of TFL1 and LFY/AP1/CAL influences flowering time and the size of the inflorescence (Ratcliffe et al., 1998, 1999). Plants that overexpress *TFL1* are late flowering, but flowering can be accelerated in 35S:*TFL1* plants by vernalization or overexpression of *FCA* (O. Ratcliffe, R. Macknight, C. Dean, and D. Bradley, personal communication). This effect is not via *FLC* because *FLC* levels are unaltered in 35S:*TFL1* plants. Interestingly, crosses between autonomous pathway mutants and *tfl1* mutants produce plants that flower later than the autonomous pathway mutants alone (Ruiz-García et al., 1997; Page et al., 1999). This finding suggests some interaction between *FLC* and *TFL1*.

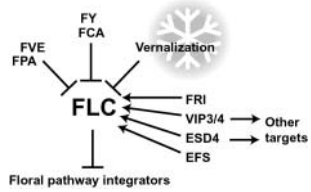

Figure 2. Pathways That Enable the Floral Transition.

A central regulator of the enabling pathways is the floral repressor FLC. High levels of FLC repress the activity of the floral pathway integrators, and this antagonizes their activation by floral promotion pathways. Many genes are involved in regulating *FLC* expression. *FCA*, *FY*, *FVE*, *FPA*, and vernalization repress *FLC* (*FLD* and *LD* are not included here because they are not required in all genotypes). *FRI*, *VIP3/4*, *ESD4*, and *EFS* upregulate *FLC*. TFL1 and SVP also may antagonize the activation of the floral pathway integrators but are not included here because their regulation and interaction with FLC are unclear.

Perhaps TFL1 affects the spatial expression of *FLC* in the meristem as it does to *LFY* and *AP1*.

SVP also acts as a floral repressor and encodes a MADS domain protein (Hartmann et al., 2000). SVP acts in a dose-dependent manner to delay flowering and does not alter the effects of photoperiod or vernalization on flowering time. *svp* mutations overcome the late flowering conferred by overexpression of *FLM*/*MAF1*, and *svp flm* double mutants behave like single mutants. Thus, *FLM*/*MAF1* and *SVP* appear to function in the same floral pathway, which interacts with the photoperiod pathway (Scortecci et al., 2003).

Recently, it was shown that two *AP2*-like genes, *TOE1* and *TOE2*, can act as floral repressors (Aukerman and Sakai, 2003). When overexpressed, *TOE1* delays flowering, and a *toe1* mutant is slightly earlier flowering than is the wild type. The *toe2* mutation does not result in an early-flowering phenotype alone, but it does enhance the *toe1* early-flowering phenotype. Both the *TOE1* and *TOE2* mRNA transcripts are targets of *EARLY ACTIVATION TAGGED* (*EAT1*), which encodes the precursor of a microRNA of the *miR172* family. Overexpression of *miR172* causes early flowering (Aukerman and Sakai, 2003; Chen, 2004) and does so by acting as a translational repressor of *AP2*-like genes (including *TOE1* and *TOE2*). In wild-type plants, *miR172* expression increases with developmental age, which makes it tempting to link the *TOE1/2–miR172* interaction with a mechanism to control flowering time based on an internal measure of age (Aukerman and Sakai, 2003).

FRIGIDA Upregulates *FLC*

FRIGIDA (*FRI*) causes upregulation of *FLC* expression, which results in Arabidopsis accessions that carry active *FRI* alleles adopting a winter annual habit—that is, they overwinter vegetatively and, after the vernalization requirement is satisfied, they flower in spring (Napp-Zinn, 1961). Allelic variation at *FRI* is a major determinant for flowering time variation in Arabidopsis accessions (Johanson et al., 2000). *FRI* increases *FLC* levels to such an extent that it overrides the influence of the strong promotive effects of long days. *FRI* encodes a novel protein containing two coiled-coil domains that suggest interaction with other proteins or nucleic acids (Johanson et al., 2000). *FRI* levels are very low at all stages of plant development, and expression is not altered by vernalization treatment (C. Lister and C. Dean, unpublished results). Furthermore, extra copies or overexpression of *FRI* do not greatly increase flowering time; thus, unlike FLC, FRI levels do not have a quantitative effect on flowering time (C. Lister and C. Dean, unpublished results). Conversely, additional copies of *FLC* in plants containing active FRI significantly delay flowering in the absence of vernalization (Michaels and Amasino, 2000).

Many early-flowering Arabidopsis accessions carry loss-of-function *FRI* alleles. Two different groups of early-flowering accessions, typified by the well-used laboratory strains L*er* and Columbia, carry different *FRI* deletion events, indicating that the early-flowering habit has arisen independently at least twice in the evolution of Arabidopsis accessions (Johanson et al., 2000). Putative loss-of-function *FRI* alleles appear to be common (Le Corre et al., 2002; Gazzani et al., 2003), and a high proportion of nonsynonymous changes in exon 1 has been detected. This extensive polymorphism may indicate positive selection for flowering time variation in natural populations (Le Corre et al., 2002).

Positive Regulators of *FLC*

Other genes that function in the upregulation of *FLC* have been identified through the analysis of early-flowering mutants that have been genetically linked to *FLC* or shown to have reduced *FLC* expression. Some of these mutants emerged from screens for mutants that flowered early in short-day photoperiods: *early flowering in short days* (*efs*) (Soppe et al., 1999), *early in short days4* (*esd4*) (Reeves et al., 2002), and *photoperiod independent early flowering1* (*pie1*) (Noh and Amasino, 2003). *ESD4* encodes a SUMO-directed protease whose targets are currently unknown (Murtas et al., 2003). *pie1* suppresses the late-flowering phenotype of both FRI and autonomous pathway mutants and is similar to ATP-dependent chromatin-remodeling proteins of the ISWI and SWI2/SNF2 family (Noh and Amasino, 2003).

Other *FLC* positive regulators have been found as suppressors of *FRI* activity and termed *vernalization independence* (*vip*) mutants (Zhang and van Nocker, 2002; Zhang et al., 2003). *vip* mutants flower earlier than *flc* null mutants and also show floral abnormalities, suggesting that *VIP* genes regulate multiple genes that influence flowering time and other developmental processes. *VIP4* exhibits sequence homology with yeast Leo1 and proteins from *Drosophila* and *Caenorhabditis elegans*. In yeast, Leo1 interacts with the ''cap'' of the proteasome and is a component of the Paf1 transcriptional complex, which is required for the full expression of a subset of yeast genes (Zhang and van Nocker, 2002). *VIP3* encodes a protein consisting almost exclusively of WD repeats and so is likely to function as a scaffold protein in protein complexes (Zhang et al., 2003).

Negative Regulation of *FLC*: Vernalization

Vernalization, the process that occurs in plants as they overwinter for many weeks in cold temperatures, strongly downregulates *FLC* levels and so accelerates flowering (Michaels and Amasino, 1999a; Sheldon et al., 1999). Vernalization is quantitative, with longer cold periods accelerating flowering more than shorter exposures, although the flowering time effect saturates after an extensive cold treatment. Vernalization is also a mitotically stable process. The cold signal is perceived many days before the meristem transition, and cuttings regenerated from vernalized plants flower without further vernalization (Metzger, 1988; Burn et al., 1993). This finding suggested that vernalization had an epigenetic basis (Wellensiek, 1962)—that is, a mitotically stable change in gene expression is established by the cold treatment that persists once the plant is returned to warmer temperatures. The vernalized state must be reset subsequently before the end of seed development to ensure that vernalization is again required for flowering in the next generation.

The molecular basis of vernalization has been explored through the isolation of mutants showing a defective vernalization response (Chandler et al., 1996; Sung and Amasino, 2004). Characterization of *FLC* RNA levels in *vernalization* (*vrn*) mutants

revealed *VRN* gene function. *FLC* decreased in the cold, but instead of remaining low during subsequent development in warm temperatures, *FLC* levels increased in *vrn1* and *vrn2* mutants (Gendall et al., 2001; Levy et al., 2002). Therefore, the main function of VRN1 and VRN2 is to maintain *FLC* repression, suggesting that they are part of the cellular machinery that provides a memory of vernalization. By contrast, *FLC* levels did not decrease in the cold in *vin3* mutants, suggesting that *VIN3* is involved in the establishment of the repression of *FLC* (Sung and Amasino, 2004).

VIN3 encodes a protein with a plant homeodomain and a fibronectin type III domain (often involved in protein–protein interactions). *VIN3* expression is induced in the later stages of cold exposure and then declines during the subsequent warm growth. Therefore, its function appears to be related to the measurement of the duration of cold exposure and in the establishment of the vernalized state (Sung and Amasino, 2004). *VRN2* encodes a protein most similar to Suppressor of zeste 12 [Su(Z)12], a Polycomb group protein required in *Drosophila* to maintain appropriate repression of certain genes (Birve et al., 2001). Polycomb group proteins usually form part of multisubunit complexes and act to maintain patterns of gene expression set up by other transcriptional regulators. Recently, Su(Z)12 was identified as part of the *EXTRA SEX COMBS*-E(Z) Polycomb complex, which maintains silent chromatin states through the methylation of specific lysine residues on histone proteins (Cao et al., 2002; Czermin et al., 2002; Müller et al., 2002). Consistent with the idea that VRN2 maintains *FLC* chromatin in a repressed state, the chromatin structure at *FLC* intron 1 in *vrn2* mutants was shown to be DNaseI hypersensitive, a condition usually associated with transcriptional activity (Gendall et al., 2001). Vernalization has been shown to induce changes in histone modifications at the *FLC* locus that are characteristic of silent chromatin, specifically an increase in histone H3 Lys-27 and Lys-9 methylation and a decrease in histone H3 acetylation (Bastow et al., 2004; Sung and Amasino, 2004). These changes in *FLC* chromatin are dependent on mutations in the vernalization pathway (Bastow et al., 2004; Sung and Amasino, 2004). Interestingly, the histone methylation changes occur in discrete regions through the *FLC* locus, some of which were in similar regions to *cis* elements required for correct *FLC* regulation as defined though in vivo expression analysis of constructs carrying *FLC* deletions (Sheldon et al., 2002). This analysis defined two promoter elements, one positive and one negative, that affected *FLC* expression in the absence of vernalization. Separate domains also were found for repression and maintenance of repression by vernalization within the large intron 1 (Sheldon et al., 2002). The exact relationship between the histone modifications and the regulatory *cis* elements has yet to be determined.

Ectopic overexpression revealed a vernalization-independent function for VRN1, predominantly through the regulation of the floral pathway integrator *FT* (Levy et al., 2002). *35S*:*VRN1* plants flowered early as a result of increased *FT* expression, whereas *FT* expression was reduced in *vrn1* mutants. *FLC* expression was unaffected in these lines until the plants had been vernalized, demonstrating that VRN1 requires vernalization-specific factors to target *FLC*. Methylation of the Lys-27 residue on histone H3 by the VRN2 complex may be the vernalization-specific process required (Bastow et al., 2004). VRN1 encodes a protein that contains two putative B3 domains (plant-specific DNA binding domains) that bind DNA in vitro in a non-sequence-specific manner (Levy et al., 2002). Whether VRN1 binds specific *FLC* sequences in vivo is still unknown. To date, little is known about the resetting of *FLC* expression. The epigenetic marks laid down during vernalization need to be erased in the gametes or developing embryo to allow high *FLC* levels in the progeny, ensuring a vernalization requirement in every generation.

FLC is not the only flowering time target of vernalization, because an *flc* null mutant grown in non-floral-promotive conditions (so that flowering time changes can be detected easily) flowers slightly earlier after vernalization (Michaels and Amasino, 2001). A likely additional target is *AGL24*, a gene with similar properties to *SOC1* (Yu et al., 2002; Michaels et al., 2003a). Like *SOC1*, *AGL24* is upregulated by vernalization, but unlike *SOC1*, this upregulation is independent of *FLC* activity. *agl24* mutants are late flowering, but this is not strongly suppressed by vernalization (Yu et al., 2002; Michaels et al., 2003a). *AGL24* appears to act downstream of *SOC1* but upstream of *LFY*, but overexpression of *AGL24* increases *SOC1* expression, indicating that the relationship between these genes may not be straightforward (Michaels et al., 2003a). The subcellular localization of AGL24 appears to be regulated by phosphorylation, although a role for this process on the effects of AGL24 on flowering time and vernalization has not been established (Fujita et al., 2003).

Negative Regulation of *FLC*: The Autonomous Pathway

fca, *fld*, *fpa*, *fve*, *fy*, *ld*, and *fld* mutants are classified as functioning in an autonomous pathway because they flower late in all photoperiods (Koornneef et al., 1991; Lee et al., 1994; Chou and Yang, 1998). Genes of the autonomous pathway function to reduce *FLC* mRNA accumulation (Michaels and Amasino, 1999a, 2001; Sheldon et al., 1999, 2000b). The late-flowering phenotype of the mutants can be overcome by either vernalization or growth in far-red-enriched light, so these environmental cues are considered to function in parallel to the autonomous pathway (Martínez-Zapater, 1990; Koornneef et al., 1991). However, unlike vernalization, far-red light does not repress *FLC* RNA levels in these mutants, indicating that vernalization and far-red floral promotion have different downstream targets (Sheldon et al., 2000a; A. Gendall and C. Dean, unpublished results).

The genes of the autonomous pathway do not function in a linear hierarchy but as a series of subgroups sharing a common target, *FLC* (Figure 2). *FCA* and *FY* form one epistatic group, with *fca* and *fy* mutants having additive effects on flowering time when combined with *fve* and *fpa* mutants (Koornneef et al., 1998) (Figure 2). *FCA* encodes a protein with two RNA binding domains and a WW protein interaction motif at the C terminus (Macknight et al., 1997). The *FCA* transcript is alternatively processed, with four detectable transcripts, only one of which produces a protein active in flowering time control (Macknight et al., 1997). *FCA* negatively regulates its own expression by promoting cleavage and polyadenylation within intron 3 (Quesada et al., 2003). This causes the production of one of the inactive forms of *FCA*

transcript at the expense of the full-length *FCA* mRNA, thus limiting the expression of active FCA. The negative autoregulation is under developmental control, requires the FCA WW interaction domain, and is dependent on FY, which interacts with FCA through the WW domain (Macknight et al., 2002; Quesada et al., 2003; Simpson et al., 2003). *FY* encodes a protein with WD repeats and a C-terminal extension carrying two PPLP motifs and belongs to a highly conserved group of eukaryotic proteins. The yeast homolog Pfs2p is an essential component of protein complexes involved in RNA 3′ end processing (Ohnacker et al., 2000). The current model is that FCA binds target RNA through its two N-terminal RNA binding domains and tethers the 3′ end–processing machinery to this RNA via interaction between the FCA WW domain and the PPLP domain of FY. One target for this interaction is *FCA* pre-mRNA, but whether *FLC* is another pre-mRNA targeted directly by FCA/FY is not yet known.

The epistasis analysis also grouped the activities of *FPA* and *FVE* in the repression of *FLC* (Koornneef et al., 1998). *FPA* also encodes an RNA binding protein (Schomburg et al., 2001), whereas *FVE* encodes a WD-repeat protein (Blázquez et al., 2001), but how they interact functionally has not been established. A *fy fpa* double mutant has not been recovered and so may be lethal (Koornneef et al., 1998), which would suggest that *FPA* and *FY* are redundant for an essential role in plant development. This is not the case for the *fve fca* double mutant, which can be recovered (Koornneef et al., 1998).

Two other genes, *LUMINIDEPENDENS* (*LD*) and *FLOWERING LOCUS D* (*FLD*), are negative regulators of FLC that show genotype-dependent effects on flowering time (Lee et al., 1994; Sanda and Amasino, 1996). *LD* encodes a homeodomain protein that is targeted to the nucleus (Lee et al., 1994). Some homeodomain proteins function in RNA processing (Dubnau and Struhl, 1996); therefore, LD also may interact with RNA as predicted for FCA and FPA (Simpson et al., 1999). *FLD* encodes a protein that is homologous with a member of a human histone deacetylase complex, which functions to remodel chromatin into a silent form via the removal of acetyl groups from histone tails (Hakimi et al., 2003; He et al., 2003). In a similar manner, FLD acts to deacetylate *FLC* chromatin, preventing the transcription of *FLC* and promoting flowering (He et al., 2003). Thus, epigenetic regulation via chromatin modification is emerging as a major mechanism to modulate *FLC* levels.

Integrating the Antagonistic Activities That Regulate *FLC*

A key question when antagonistic pathways control the expression of a common target is how the predominance of those activities is exerted. With respect to *FLC*, FRI activates the expression and overcomes the repression caused by genes of the autonomous pathway. This predominance may be attributable to differing temporal expression of the genes. Expression of *FCA* is maintained at low levels during early seedling development as a result of *FCA* negative autoregulation promoting the use of an internal polyadenylation site within *FCA* intron 3 (Quesada et al., 2003). However, as judged by levels of an *FCA*:β-glucuronidase (*GUS*) translational fusion, which specifically monitors when an active transcript is generated, FCA levels increase in the shoot and root meristematic regions by ~4 to 5 days after germination (Macknight et al., 2002). This may reflect a specific developmental mechanism to increase FCA levels and thus decrease *FLC* levels in the meristem at later stages of development. Removal of introns from *FCA* bypasses the autoregulation and results in increased levels of FCA much earlier in development. This overcomes the repression of flowering conferred by *FRI* (Quesada et al., 2003). Thus, altering the timing or levels of FCA and FRI accumulation can reverse the outcome of their antagonistic activities. Therefore, the negative autoregulation of *FCA* may have evolved to limit FCA activity early in development and hence avoid precocious flowering too soon after germination.

PATHWAYS THAT PROMOTE THE FLORAL TRANSITION

A variety of environmental and endogenous signals act to promote the floral transition through the activation of the floral pathway integrators (Figure 3). Our understanding of the floral promotion pathways is most advanced for the environmental cue photoperiod, but recent work has revealed the existence of a separate floral pathway involving light quality that is possibly closely connected to ambient temperature effects (Blázquez et al., 2003; Cerdan and Chory, 2003; Halliday et al., 2003). Our understanding of endogenous promoters of flowering is limited still, but this should be a fertile area of research in the future.

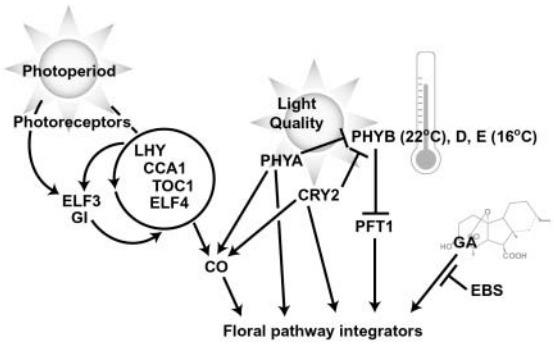

Figure 3. Pathways That Promote the Floral Transition.

Photoperiod, light quality, and GAs promote the floral transition by activating the floral pathway integrators. The photoperiod pathway promotes flowering under long days. The photoreceptors perceive light and, along with entrainment factors, such as ELF3 and GI, synchronize the circadian oscillator (CCA1, LHY, TOC1, and ELF4) with the environment. Changes in daylength are detected by this entrained circadian system and transduced into flowering time information via cyclic expression of *CO*. Under short days, *CO* expression coincides with the night, whereas under long days, expression occurs during the day. Light activation of CO by PHYA and CRY2 leads to an increase in *FT* expression and the promotion of flowering. Light quality also acts independently of the photoperiodic pathway. The red light photoreceptors PHYB, PHYD, and PHYE repress flowering. At 22°C, PHYB is the predominant photoreceptor mediating this response, whereas at 16°C, this role is taken by PHYE. The photoreceptors CRY2 and PHYA act to promote flowering either directly or by repressing PHYB. The hormone GA promotes flowering under short days by regulating the expression of the floral integrators, and EBS represses this effect at least on *FT*.

Photoperiod Pathway

To perceive and respond to changes in photoperiod, plants must be able to detect light duration (period) and couple this to an internal timer or oscillator (circadian clock) (Thomas and Vince-Prue, 1997). In Arabidopsis, the red/far-red light–absorbing phytochromes (Quail, 2002) and the UV/blue light–absorbing cryptochromes (Lin, 2000) are the main photoreceptors involved in daylength sensing. Light perceived by these photoreceptors acts to reset the oscillator so that it remains in synchrony (entrained) with its environment. Entrainment is achieved via a number of mechanisms, one of which involves PHYTOCHROME-INTERACTING FACTOR 3 (PIF3), a basic helix-loop-helix transcription factor. PHTYOCHROME (PHY) B is activated by light and then binds to PIF3, resulting in the upregulation of both CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), two proteins postulated to be constituents of the central oscillator (Martínez-García et al., 2000). Other genes that influence entrainment and flowering time include the ZTL/FKF/LKP family (Nelson et al., 2000; Somers et al., 2000; Schultz et al., 2001). *zeitlupe* (*ztl*) mutants are late flowering in long days and disrupt circadian rhythms, causing an increase in period length. This phenotype is strongly dependent on light intensity, suggesting that ZTL may act as a photoreceptor (Somers et al., 2000). Overexpression of LOV KELCH PROTEIN2 (*LKP2*) induces late flowering in long days and abolishes a number of circadian rhythms (Schultz et al., 2001). The third member, *FLAVIN BINDING KELCH REPEAT F-BOX1* (*FKF1*), causes late flowering when mutated but has less influence on the circadian system (Nelson et al., 2000). These three genes encode proteins that contain a PAS domain, an F-box (involved in protein degradation), six repeated kelch motifs (a domain involved in protein–protein interactions), and a LOV domain (a light-sensing domain of the blue light receptor phototrophin). It has been suggested that the presence of these motifs, along with protein interactions in vitro between ZTL, PHYB, and CRYPTOCHROME1 (CRY1), indicates that the ZTL/FKF/LKP2 family functions to recruit and degrade clock components in a light-dependent manner (Jarillo et al., 2001; Kim et al., 2003). However, recent studies of the LOV domain of FKF1 have shown that ZTL, FKF1, and LKP2 form a new family of blue light photoreceptors, suggesting that these proteins function in a unique light-signaling pathway (Imaizumi et al., 2003).

EARLY FLOWERING3 (ELF3) also is a component of the signal transduction pathway between the photoreceptors and the oscillator. It was identified originally as an early-flowering mutant that disrupted the circadian system and caused arrhythmia, but only under constant light conditions (Hicks et al., 1996). The reason for this light-conditional arrhythmia has been explained by a number of recent studies. *ELF3* has been found to encode a nuclear protein whose expression is regulated in a circadian manner with a peak at the beginning of the night (Covington et al., 2001; Liu et al., 2001). The presence of ELF3 during the night period has been shown to antagonize light input to the oscillator and the circadian-regulated *CAB* gene. Therefore, it has been proposed that ELF3 sustains rhythmicity in constant light by repressing phototransduction at subjective dusk (McWatters et al., 2000). In the *elf3* mutant, the absence of ELF3 under constant light exposes the oscillator to light signaling during the subjective night phase, causing the oscillator to stop. Therefore, ELF3's effects on the photoperiodic regulation of flowering time result from its role in the circadian system. Exactly how ELF3 functions in repressing light signaling is unknown, but it does exhibit a number of *phyB*-like phenotypes and has been shown to interact physically with PHYB (Liu et al., 2001). Aberrant PHYB-mediated responses also are observed in the *sensitivity to red light reduced1* (*srr1*) mutant. Like *elf3*, *srr1* mutants are pale with elongated petioles and hypocotyls and are early flowering (Staiger et al., 2003). The late-flowering mutant *gigantea* (*gi*) is also implicated in the PHYB pathway, and an allele of *GI* was isolated from a screen for inhibitors of hypocotyl elongation in red light, a PHYB-dependent phenotype (Huq et al., 2000). In addition to these *phyB* effects, *gi* and *srr1* mutants disrupt the circadian system, shortening the period and reducing the amplitude of a number of circadian outputs (Huq et al., 2000; Staiger et al., 2003). Together, these data indicate that ELF3, SRR1, and GI function downstream of PHYB and act to maintain the viability of the circadian system so that it generates the correct period and amplitude. Therefore, when mutated, these genes disrupt the circadian system and consequently cause aberrant regulation of the photoperiodic timing of the floral transition.

CCA1, LHY, and TIMING OF CAB EXPRESSION1 (TOC1) have been identified as possible candidates for oscillator components in Arabidopsis (Millar et al., 1995; Schaffer et al., 1998; Wang and Tobin, 1998) (Figure 3). When mutated, *TOC1*, *CCA1*, and *LHY* all confer early flowering. *CCA1* and *LHY* both encode very similar proteins with a single MYB repeat. The expression of *CCA1* and *LHY* RNA and protein is regulated by the circadian clock, with a peak in the early morning. Mutations in either gene shorten the circadian period, whereas overexpression of either one results in downregulation of the other and general arrhythmia (Schaffer et al., 1998; Wang and Tobin, 1998; Green and Tobin, 1999; Mizoguchi et al., 2002). *TOC1* encodes a pseudoresponse regulator whose expression also is regulated by the circadian clock, with a peak of expression in the evening. Mutations in *TOC1* shorten the period of circadian outputs, and overexpression causes arrhythmia (Somers et al., 1998; Strayer et al., 2000).

From the expression profiles of *LHY*, *CCA1*, and *TOC1*, a model has been proposed whereby these three proteins function to produce an autoregulatory transcriptional and translational negative feedback loop (Alabadí et al., 2001). During the late evening, TOC1 activates *CCA1* and *LHY*, resulting in a peak of expression at the start of the day. Subsequent increases in CCA1 and LHY during the day act to repress *TOC1* expression; consequently, *CCA1* and *LHY* mRNA levels decay by the end of the day as a result of the removal of their activator, TOC1. Reduction in CCA1 and LHY relieves the repression of *TOC1*, and the cycle begins again, forming an oscillatory loop. However, it must be stated that many aspects of this model have yet to be proven, which has led to the development of an alternative model in which the members of the TOC1 family form the circadian oscillator (Makino et al., 2002; Matsushika et al., 2002a, 2002b). This model involves the sequential expression of a quintet of *TOC1*-like genes in circadian waves, independent of the entrained photoperiod conditions (Matsushika et al., 2000). The expression profile of these genes changes constantly throughout the 24-h cycle, allowing some indication of the progression of time. Recent data have

demonstrated that ELF4 also may be involved in the oscillator. *elf4* mutants cause arrhythmia and, like *toc1-2*, cause the downregulation of *CCA1* mRNA. ELF4 expression peaks during the night, suggesting that it may function along with TOC1 to activate *CCA1* and *LHY* expression (Doyle et al., 2002).

The *CONSTANS* (*CO*) gene plays a key role in integrating light and temporal information essential for determining how daylength sensing is achieved. *CO* encodes a transcription factor with two B-box zinc fingers and directly activates *FT*. *co* mutants flower late under long days, but flowering time is unaffected in short days. Overexpression of *CO* causes early flowering under either photoperiod (Putterill et al., 1995; Suárez-López et al., 2001). Altered levels of *CO* expression are observed in a number of circadian mutants. In late-flowering, gain-of-function *lhy* and *cca1* mutants, *CO* mRNA levels are low, whereas in the early-flowering *elf3* mutant, *CO* levels are high. The effects of these mutations on the circadian clock appear to be translated into a flowering effect via CO, an idea that is consistent with the circadian regulation of *CO* expression. *CO* mRNA also is modulated by photoperiod, and under short days, *CO* expression peaks in the night. Under long days, there is a peak in expression at the end of the day, an expression profile that is mirrored by *FT*. Therefore, a model has been proposed whereby *CO* expression provides a light-sensitive rhythm for the perception of daylength. Only under long days would high *CO* mRNA levels coincide with light, leading to an increase in *FT* expression (Suárez-López et al., 2001). This description of *CO* function provides molecular support for the theoretical photoperiodic model of external coincidence, first proposed by Bünning (1936). This model predicts that the circadian clock generates a photoperiodic response rhythm that is sensitive to light at certain phases of the cycle. When light coincides with these sensitive phases, a photoperiodic response results. In Arabidopsis, the photoperiodic response rhythm is provided for by the rhythm of *CO* expression, and light input to FT is perceived by PHYA and CRY2 (Yanovsky and Kay, 2002). In long-day conditions, *CO* expression coincides with the light input, resulting in the activation of *FT* expression and the promotion of flowering. Despite these advances in understanding the photoperiod regulation of flowering, the mechanism that generates the daylength-dependent expression profiles of *CO* remains unclear. However, recent studies suggest that FKF1 may be involved (Imaizumi et al., 2003). It is interesting that PHYA and CRY2 are light-labile proteins that exhibit a diurnal pattern in protein abundance under short days but not long days. This may act as part of the daylength-sensing mechanism (Mockler et al., 2003). The importance of protein stability in flowering time has been shown through quantitative trait loci analysis. In the Arabidopsis accession Cvi, a single amino acid substitution causes an increased stability of CRY2-Cvi, and this allele confers an early-flowering phenotype in short days (El-Assal et al., 2001).

Integrating the Antagonistic Activities of the Photoperiod Pathway and *FLC* on the Expression of Floral Pathway Integrators

The photoperiod and FLC-regulated pathways ultimately converge on a few targets that include FT and SOC1. The molecular basis of the antagonistic action between the two pathways has been investigated by studying the levels of these integrator genes in a number of mutant combinations. For example, the activation of *SOC1* by overexpression of *CO* was blocked completely by overexpression of *FLC* (Hepworth et al., 2002), whereas the photoperiod deficiency in *co* and *gi* mutants was partially restored when combined with increased levels of *FLC* generated by *fca* (Koornneef et al., 1998). In a similar manner, the daylength-insensitive phenotype of CRY-Cvi can be overcome by the presence of high levels of FLC, which decrease the levels of CRY2 and restore photoperiod response (El-Assal et al., 2003). To determine how the interactions between these pathways are achieved, the *cis* elements involved in *SOC1* repression by FLC and activation by CO were examined in a deletion series of *SOC1* promoter:GUS fusions in transgenic plants. A 351-bp promoter sequence was defined that mediates both activation by CO and repression by FLC. A MADS box binding element (CArG) is present within the 351-bp region, and this was shown to bind FLC in vitro (Hepworth et al., 2002). Thus, when both CO and FLC proteins are present, FLC binds to the 351-bp region in vivo, possibly at the CArG element, impairing the functioning of CO in the activation of *SOC1* transcription (Hepworth et al., 2002). In addition, high levels of *FLC* cause the downregulation of *CRY2*, which prevents the CRY2 promotion of *CO* (El-Assal et al., 2003). Thus, FLC acts at several levels to downregulate the effects of the photoperiod promotive pathway.

Light Quality and Ambient Temperature

Light also affects flowering time independently of photoperiod via a light quality pathway (Simpson and Dean, 2002). Light quality is affected by shading, which results in a reduction in the ratio of red to far-red light. PHYB represses flowering via the downregulation of *FT* (Halliday et al., 2003), and this is mediated by *PHYTOCHROME AND FLOWERING TIME1*, a gene that encodes a nuclear protein that activates *FT* expression (Cerdan and Chory, 2003). PHYD and PHYE also repress flowering, but their effects are observed only when the predominant effect of PHYB is absent (Halliday et al., 1994; Aukerman et al., 1997; Devlin et al., 1998, 1999). In contrast to red light, far-red and blue light promote flowering. *phyA* is late flowering in conditions in which the light given at the end of the light period is far-red enriched or when the night period is interrupted by a short light period (Reed et al., 1994), and *cry2* is late flowering under long days (Lin, 2000). It has been proposed that CRY2 and PHYA promote flowering via two independent pathways, one directly on floral pathway integrators and one by repression of PHYB (Mockler et al., 2003). Therefore, the CRY2 and PHYA pathways act in an antagonistic manner to the PHYB pathway, forming a network that functions to regulate flowering under varying light qualities, providing a readout to the plant of how crowded the local environment is with other plants.

Ambient temperature significantly influences the phenotypic effects caused by the loss of different photoreceptors. *phyB* flowers earlier than the wild type at 22°C, whereas at 16°C, the mutation has no effect (Halliday et al., 2003). At 16°C, PHYE appears to act as the predominant phytochrome regulating flowering: *phyA phyB phyD phyE* quadruple mutants flower

earlier than *phyA phyB phyD* triple mutants, an effect that is mediated by *FT* levels (Halliday et al., 2003). The effect of the *cry2* mutation on flowering also is temperature sensitive. At 23°C *cry2* mutations delay flowering, but this effect is enhanced greatly at 16°C. The increased delay in flowering appears to be caused by the loss of PHYA activity at 16°C. At 23°C, PHYA functions redundantly with CRY2 to promote flowering, so *cry2* mutants are only slightly delayed. However, at 16°C, *cry2* is as late flowering as the *phyA cry2* double mutant at 23°C, suggesting a loss of PHYA activity at the lower temperature (Blázquez et al., 2003).

The role of ambient temperature on flowering time control also has been analyzed in the wild type and flowering time mutants (Blázquez et al., 2003). The flowering of wild-type plants is delayed at 16°C, although *fca* and *fve* mutants show little difference in flowering time at 16 or 23°C. FCA and FVE are thought to mediate a temperature-dependent repression of *FT* expression that does not involve *FLC*, the usual target of these genes within the pathways that enable the floral transition (Blázquez et al., 2003). This has been interpreted as showing that *FCA* and *FVE* function in a pathway that mediates ambient temperature effects, in addition to their role in the autonomous pathway. However, it is possible that this is just a side effect of those mutants containing high levels of FLC, which will effectively suppress *FT* expression, ameliorating any regulation by the light quality pathway. The effects of ambient temperature on flowering may all be through the light quality pathway activation of *FT* and may not directly involve *FCA* or *FVE* function.

Hormonal Inputs

Genetic studies have confirmed the physiological findings that gibberellins (GAs) accelerate Arabidopsis flowering (Langridge, 1957). Plants overexpressing GA-20 oxidase, a gene late in the GA biosynthesis pathway, are early flowering in both long days and short days (Huang et al., 1998; Coles et al., 1999). The *spindly* mutation is considered to exhibit constitutively active GA signaling and is early flowering (Jacobsen and Olszewski, 1993), whereas plants overexpressing *FLOWERING PROMOTIVE FACTOR1*, a gene involved in GA signal transduction or responsiveness to GAs, also flower early (Kania et al., 1997). Conversely, a decrease in GA levels or insensitivity to GA signaling delays flowering, although this effect is significant only in short days (Wilson et al., 1992). For example, a mutation in *GA1*, the first committed step in GA biosynthesis, makes Arabidopsis an obligate long-day plant because it no longer flowers under short days. *gai* mutants are insensitive to GAs and also are late flowering in short days (Wilson et al., 1992). Double mutant analyses have established that the GA pathway is genetically distinct from the photoperiod promoting pathway and the enabling pathway (Figure 3) and have confirmed that the GA pathway has less influence on flowering time in long days than in short days (Putterill et al., 1995; Chandler et al., 2000; Reeves and Coupland, 2001). However, in the absence of the long-day promotion pathway, the GA pathway is an important promoter of flowering (Reeves and Coupland, 2001). Some physiological experiments have suggested that vernalization works via the GA pathway, and in some plant species, GA application can substitute for a vernalization treatment (Zeevaart, 1983; Sheldon et al., 1999). However, in Arabidopsis, vernalization and GA action are clearly independent, because the *fca-1 ga1-3* and *FRI FLC ga1-3* genotypes, in which GA biosynthesis is blocked, still respond to vernalization (Michaels and Amasino, 1999b; Chandler et al., 2000).

How changes in GA biosynthesis or signal transduction result in altered flowering time is an area of active research. One target of the GA signal is *LFY*, because *LFY* promoter activity is reduced in a *ga1-3* mutant and increased by exogenous GA application in both the wild type and *ga1-3* (Blázquez et al., 1998). The promoter region of *LFY*, which is responsible for GA-induced expression, was found to include an 8-bp motif that is a consensus binding site sequence for MYB transcription factors (Blázquez and Weigel, 2000). A MYB-like transcription factor, *AtMYB33*, has an overlapping expression pattern with *LFY* in the floral meristem and can bind the 8-bp *LFY* promoter motif in vitro (Gocal et al., 2001). *AtMYB33* expression is induced upon GA application, suggesting that it may play a role in the GA responsiveness of the *LFY* promoter, so GAs may alter flowering time by increasing *LFY* expression. The phenotype of the *atmyb33* mutant has not been reported. Constitutive expression of *LFY* accelerates flowering but cannot fully complement the flowering time effect of *ga1-3* (Blázquez et al., 1998). This may be attributable to another floral integrator, *SOC1*, also being regulated by GAs (Moon et al., 2003a). The study of a mutation in *EARLY BOLTING IN SHORT DAYS* (*EBS*) suggests that GAs also may influence *FT* expression (Gómez-Mena et al., 2001; Piñeiro et al., 2003). *EBS* encodes a protein with a bromoadjacent homology domain and a plant homeodomain zinc finger and so is assumed to be part of a chromatin-remodeling complex (Piñeiro et al., 2003). *ebs* mutants are significantly early flowering in short days and slightly early flowering in long days, but the early-flowering phenotype is lost in *ebs ga1-3* and *ebs ft* double mutants (Gómez-Mena et al., 2001; Piñeiro et al., 2003). This finding suggests that GAs can alter *FT* expression patterns but that this effect is repressed by EBS via chromatin remodeling. Therefore, the inability of *LFY* overexpression to fully complement the late-flowering phenotype of *ga1-3* may be attributable to the altered expression of *SOC1* and/or *FT* in *ga1-3*.

There is considerable research currently analyzing the *cis* elements in *FT*, *SOC1*, and *LFY* that mediate the convergence of the GA, photoperiod, and light quality activation pathways. The *cis* elements through which GAs activate the *LFY* promoter are distinct from those required for photoperiodic activation, demonstrating that at least some of the convergence of these different promotion pathways occurs at the level of the *LFY* promoter (Blázquez and Weigel, 2000).

Other hormones are implicated in the control of flowering time in Arabidopsis. Mutants that reduce abscisic acid biosynthesis are earlier flowering under noninductive conditions, suggesting that abscisic acid inhibits flowering (Martínez-Zapater et al., 1994). In support of this, *abi1* and *abi2* (abscisic acid signaling mutants) have been shown to reduce the flowering time of *fca-1* mutants (Chandler et al., 2000). Despite the large amount of physiological evidence implicating cytokinins in flowering time control (Bernier et al., 1993), genetic evidence for a role for cytokinins in the promotion of Arabidopsis flowering is lacking. Ethylene signaling mutants (Guzmán and Ecker, 1990; Ogawara

et al., 2003), the brassinosteroid biosynthesis mutant *det2* (Chory et al., 1991), and plants altered in salicylic acid biosynthesis (Martínez et al., 2004) are late flowering, implicating these plant growth regulators in floral promotion pathways.

REPRESSING THE FLORAL TRANSITION OR RESETTING EXPRESSION IN THE NEXT GENERATION?

In addition to the pathways described above, which specifically converge on the regulation of the floral pathway integrators, there is an increasing list of genes that have been classified as floral repressors based on their mutant phenotypes being early flowering. These have been difficult to group into genetic pathways, but they have been characterized as ectopically expressing genes normally expressed in the floral state and repressed in the vegetative state (reviewed by Sung et al., 2003). This class of floral repressors has led to the idea that in the absence of other regulators, flowering is the default developmental pathway and vegetative development is maintained for a distinct period through the active repression of genes normally expressed in the floral state. A period of vegetative growth is thought to be necessary so that plants can accumulate sufficient reserves before they embark on the energy-consuming process of flowering and seed production.

An alternative function of these genes, however, might be to reset gene expression states for the next generation or to maintain these ''reset'' global expression states. A key difference with animal development is that in plants no cells are laid down early in development to form a distinct germ line. Instead, the cells that produce gametes are derived from cells within the flower whose relatively recent predecessors expressed floral-specific genes such as those that determine floral organ identity. There must be global resetting of gene expression to erase the epigenetic marks established during the differentiation of the flower, before the embryo is produced and perhaps even before the final gamete is formed. If these floral-specific genes remained expressed in the gametes and embryo, then the floral state would be expressed immediately after germination.

Extreme examples of early-flowering mutants that would fit this model are *embryonic flower1* (*emf1*) and *emf2*. These mutants are described as bypassing vegetative growth and forming flowers (albeit very abnormal ones) immediately after germination (Sung et al., 1992). Both *emf* mutants show increased expression of many genes involved in floral organ identity (Chen et al., 1997; Moon et al., 2003b). Additionally, genes involved in seed maturation are upregulated in *emf1* but not in *emf2* (Moon et al., 2003b), showing that gene expression/repression is altered globally in *emf1*. *EMF1* encodes a novel protein predicted to be a transcription factor (Aubert et al., 2001), and EMF2 is a homolog of VRN2 and so likely to play a role in the maintenance of chromatin states (Yoshida et al., 2001).

Another gene likely to be involved in resetting and/or the maintenance of resetting is *FERTILIZATION-INDEPENDENT EMBRYO* (*FIE*), initially described as being required for the repression of endosperm development (Ohad et al., 1996). *FIE* is highly similar to the *EXTRA SEX COMBS* gene from *Drosophila*, which encodes a WD Polycomb group protein that acts as a molecular scaffold in protein complexes involved in the maintenance of chromatin states (Ohad et al., 1999). *FIE* is expressed throughout the plant, but because *fie* mutations are lethal, it was initially not possible to investigate *FIE* function later in development (Ohad et al., 1996, 1999). Using a modified *FIE* construct that ceases to express *FIE* early in seed development, *fie* homozygous seedlings were obtained (Kinoshita et al., 2001). These plants produced flowers immediately after germination, resembled *emf* mutants, and ectopically expressed *LFY* and the floral homeotic genes *AGAMOUS* (*AG*) and *PISTILLATA* (*PI*) during early embryogenesis. The fact that EMF2 and FIE are homologs of chromatin-remodeling factors suggests that resetting occurs predominantly at the chromatin level.

fwa mutants, which are dominant for late flowering, might have been considered as putative resetting mutants given that *FWA* expression was shown to be limited to siliques and germinating seedlings in wild-type plants (Soppe et al., 2000). However, recent work has shown that *FWA* displays imprinted (maternal-origin specific) expression in endosperm and so is unlikely to play a role in resetting gene expression states in the embryo (Kinoshita et al., 2003).

Other early-flowering mutants may define genes with more general roles in the maintenance of silenced states of gene expression. The *like-heterochromatin protein/terminal flower2* (*lhp1/tfl2*) mutant has an early-flowering phenotype and produces a small inflorescence because of the early production of a terminal flower (Gaudin et al., 2001; Kotake et al., 2003). LHP1 is a homolog of *Drosophila* HETEROCHROMATIN PROTEIN1 (HP1), a well-conserved protein associated with maintaining silent chromatin states. *lhp1/tfl2* was shown to have increased levels of *FT*, which accounted for the early-flowering phenotype (Kotake et al., 2003; Takada and Goto, 2003). *AP3*, *PI*, *AG*, and *SEPALLATA3* were expressed at higher levels in the flowers, although floral organ development was not altered (Kotake et al., 2003). A central role of LHP1 may be in the maintenance of *FT* repression after resetting in the gametes/embryo. The less extreme phenotype of *lhp1/tfl2* compared with *emf* mutants and *fie* may be attributable to the requirement for activators (at least CO) as well as the loss of repression to achieve levels of *FT* that are able to accelerate flowering. *CURLY LEAF* (*CLF*) encodes a SET domain Polycomb group protein and is required for the

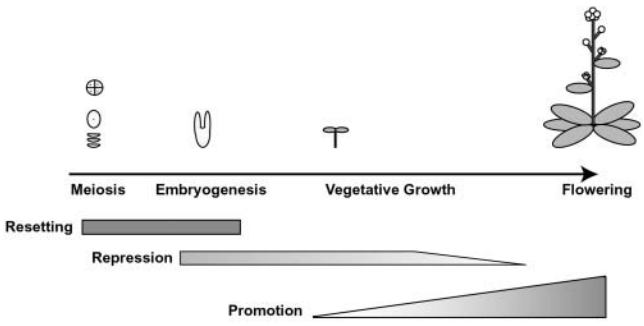

Figure 4. Resetting, Repression, and Promotion Phases in the Life Cycle.

The plant life cycle can be viewed in three sequential floral phases: resetting of global gene expression patterns, and specific repression and promotion of floral pathway integrators.

stable repression of *AG* (Goodrich et al., 1997). The *clf* mutant has an early-flowering phenotype that is largely caused by the ectopic expression of *AG*, as assessed by flowering time analysis of a *clf ag* double mutant. The ectopic expression of *AG* may be derived from the lack of resetting of *AG* from the previous generation.

In addition, specific floral repressors may exist that act to maintain the vegetative state. Global gene expression analysis using microarrays revealed a large group of potential floral repressors downregulated upon the transfer of Arabidopsis plants from short-day to long-day conditions. These included the two AP2 domain–encoding genes that can repress flowering, *SCHLAFMUTZE* and *SCHNARCHZAPFEN* (Schmid et al., 2003), which like TOE1/2 may be regulated by microRNAs (Schmid et al., 2003).

SUMMARY

In this review, the network of pathways that controls the timing of the transition to flowering has been divided into those that enable and those that promote the floral transition (Figure 4). The interaction of these different pathways changes in response to different environmental and endogenous cues to generate the plasticity and diversity of the flowering response. The predominance of the different floral pathways also must change over the life cycle of the plant. During early vegetative development, floral repressors in the enabling pathways overcome any promotive cues, ensuring that a sufficiently long vegetative phase occurs for the necessary energy reserves to be accumulated. During the later stages of vegetative development, the activity of the floral repressors declines and there is a progressive activation of floral promoters until a quantitative threshold is reached and the transition of the meristem from a vegetative to a reproductive state occurs. The pattern of gene expression in the flowers then must be reset in the gametes and developing embryos so that the next generation can determine its own ''right time to flower.'' Therefore, the plant life cycle can be viewed in three sequential floral phases resulting from the changing predominance of three activities: resetting, repression, and promotion (Figure 4). Judging from how fast our knowledge has progressed in the last few years, progress in determining the molecular basis of how these different phases are regulated and maintained should be rapid.

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